This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A method of identifying an agent that alters mitochondrial function, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a cell containing cytosol, a mitochondrion and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the cytosol;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity, wherein a decrease over time in the signal that is proportional to the level of calcium in the cytosol indicates mitochondrial calcium uniporter activity, and (ii) mitochondrial uncoupler or respiratory inhibitor activity, wherein an increase over time in the signal that is proportional to the level of calcium in the cytosol without repeating step (a) indicates mitochondrial uncoupler or respiratory activity; and

(c) comparing the signal generated by the calcium indicator molecule at one or more of said time points in the absence of a candidate agent, to the signal generated by the calcium indicator molecule at one or more of said time points in the presence of the candidate agent, and therefrom identifying an agent that alters mitochondrial function.

2. (Original) The method of claim 1 wherein the step of contacting is repeated at least once.

3. (Original) The method of claim 1 wherein the sample contains at least one compound that alters intracellular distribution of a calcium cation.

4. (Original) The method of claim 3 wherein the compound that alters intracellular calcium cation distribution is selected from the group consisting of thapsigargin and Ru360.

5. (Original) The method of claim 3 wherein the compound that alters intracellular calcium cation distribution is selected from the group consisting of a calcium ionophore and a membrane permeable compound that alters intracellular calcium distribution.

6. (Original) The method of claim 3 wherein the sample contains at least one compound that uncouples oxidative phosphorylation from ATP production.

7. (Original) The method of claim 1 wherein the candidate agent is membrane permeable.

8. (Original) The method of claim 1 wherein the calcium indicator molecule is membrane permeable.

9. (Original) The method of claim 1 wherein the source of calcium cations is exogenous to the cell.

10. (Original) The method of claim 1 wherein the sample contains at least one compound that uncouples oxidative phosphorylation from ATP production.

11. (Original) The method of claim 1 wherein the cell comprises at least one polypeptide that is a Bcl-2 family member.

12. (Original) The method of claim 1 wherein the cell expresses a gene encoding a polypeptide that regulates cytosolic calcium.

13. (Original) The method of claim 12 wherein the gene encodes a mitochondrial calcium uniporter.

14. (Original) The method of claim 12 wherein the gene is a transfected gene.

15. (Original) The method of claim 14 wherein the gene encodes a mitochondrial calcium uniporter.

16. (Original) The method of claim 1 wherein the cell is a permeabilized cell.

17. (Original) The method of claim 1 wherein the cell adheres to a solid substrate.

18. (Original) The method of claim 1 wherein the cell is a non-adherent cell.

19. (Currently Amended) A method of identifying an agent that uncouples oxidative phosphorylation from ATP production, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a cell containing cytosol, a mitochondrion and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the cytosol;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity, wherein a decrease over time in the signal that is proportional to the level of calcium in the cytosol indicates mitochondrial calcium uniporter activity, and (ii) mitochondrial uncoupler or respiratory inhibitor activity, wherein an increase over time in the signal that is proportional to the level of calcium in the cytosol without repeating step (a) indicates mitochondrial uncoupler or respiratory activity;

(c) repeating steps (a) and (b) at least once; and

(d) comparing (i) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the absence of the candidate agent, to (ii) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the presence of the candidate agent, wherein an increased level of calcium in the cytosol at a time point prior to a contacting step in the presence of the agent, compared to the level of calcium in the cytosol prior to a contacting step in the absence of the agent, indicates an agent that uncouples oxidative phosphorylation from ATP production.

20. (Currently Amended) A method of identifying an agent that that is a respiratory inhibitor, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a cell containing cytosol, a mitochondrion and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the cytosol;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity, wherein a decrease over time in the signal that is proportional to the level of calcium in the cytosol indicates mitochondrial calcium uniporter activity, and (ii) mitochondrial uncoupler or respiratory inhibitor activity, wherein an increase over time in the signal that is proportional to the level of calcium in the cytosol without repeating step (a) indicates mitochondrial uncoupler or respiratory activity;

(c) repeating steps (a) and (b) at least once; and

(d) comparing (i) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the absence of the candidate agent, to (ii) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the presence of the candidate agent, wherein an increased level of calcium in the cytosol at a time point prior to a contacting step in the presence of the agent, compared to the level of calcium in the cytosol prior to a contacting step in the absence of the agent, indicates an agent that is a respiratory inhibitor.

21. (Currently Amended) A method of identifying an agent that alters a mitochondrial calcium uniporter, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a cell containing cytosol, a mitochondrion and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the cytosol;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity, wherein a decrease over time in the signal that is

proportional to the level of calcium in the cytosol indicates mitochondrial calcium uniporter activity, and (ii) mitochondrial uncoupler or respiratory inhibitor activity, wherein an increase over time in the signal that is proportional to the level of calcium in the cytosol without repeating step (a) indicates mitochondrial uncoupler or respiratory activity;

(c) repeating steps (a) and (b) at least once; and

(d) comparing (i) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the absence of the candidate agent, to (ii) the signal generated by the calcium indicator molecule at one or more of said time points prior to and following at least one of the contacting steps in the presence of the candidate agent, wherein an increased level of calcium in the cytosol at a time point following a contacting step in the presence of the agent, compared to the level of calcium in the cytosol following a contacting step in the absence of the agent, indicates that the agent alters a mitochondrial calcium uniporter.

Claims 22-42 (Canceled)

43. (Currently Amended) A method of identifying an agent that alters mitochondrial function, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a cell containing a mitochondrion, cytosol and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, and wherein the calcium indicator molecule is membrane permeable and capable of generating a detectable signal that is proportional to the level of calcium in the cytosol, with

(ii) a calcium ionophore, under conditions and for a time sufficient to increase calcium levels within the cell;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity, wherein a decrease over time in the signal that is

proportional to the level of calcium in the cytosol indicates mitochondrial calcium uniporter activity, and (ii) mitochondrial uncoupler or respiratory inhibitor activity, wherein an increase over time in the signal that is proportional to the level of calcium in the cytosol without repeating step (a) indicates mitochondrial uncoupler or respiratory activity; and

(c) comparing the signal generated by the calcium indicator molecule at one or more of said time points in the absence of the candidate agent, to the signal generated by the calcium indicator molecule at one or more of said time points in the presence of the candidate agent, and therefrom identifying an agent that alters mitochondrial function.

44. (Original) The method of claim 43 wherein the calcium ionophore is selected from the group consisting of ionomycin, A23187, NMDA and a cell depolarization signal.

45. (Original) The method of claim 43 wherein the step of contacting is repeated at least once.

46. (Original) The method of claim 43 wherein the sample contains at least one compound that alters intracellular distribution of a calcium cation.

47. (Original) The method of claim 46 wherein the compound that alters intracellular calcium cation distribution is selected from the group consisting of thapsigargin and Ru360.

48. (Original) The method of claim 46 wherein the compound that alters intracellular calcium cation distribution is selected from the group consisting of a calcium ionophore and a membrane permeable compound that alters intracellular calcium distribution.

49. (Original) The method of claim 46 wherein the sample contains at least one compound that uncouples oxidative phosphorylation from ATP production.

50. (Original) The method of claim 43 wherein the candidate agent is membrane permeable.

51. (Original) The method of claim 43 wherein the source of calcium cations is exogenous to the cell.

52. (Original) The method of claim 43 wherein the sample contains at least one compound that uncouples oxidative phosphorylation from ATP production.

53. (Original) The method of claim 43 wherein the cell comprises at least one polypeptide that is a Bcl-2 family member.

54. (Original) The method of claim 43 wherein the cell expresses a gene encoding a polypeptide that regulates cytosolic calcium.

55. (Original) The method of claim 54 wherein the gene encodes a mitochondrial calcium uniporter.

56. (Original) The method of claim 54 wherein the gene is a transfected gene.

57. (Original) The method of claim 56 wherein the gene encodes a mitochondrial calcium uniporter.

58. (Original) The method of claim 43 wherein the cell adheres to a solid substrate.

59. (Original) The method of claim 43 wherein the cell is a non-adherent cell.

Claims 60-63 (Canceled)

64. (Currently Amended) A method of identifying an agent that alters mitochondrial function, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising a permeabilized cell depleted of cytosol, a mitochondrion and a calcium indicator molecule, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the cell;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity, wherein a decrease over time in the signal that is proportional to the level of calcium in the cytosol indicates mitochondrial calcium uniporter activity, and (ii) mitochondrial uncoupler or respiratory inhibitor activity, wherein an increase over time in the signal that is proportional to the level of calcium in the cytosol without repeating step (a) indicates mitochondrial uncoupler or respiratory activity; and

(c) comparing the signal generated by the calcium indicator molecule at one or more of said time points in the absence of a candidate agent, to the signal generated by the calcium indicator molecule at one or more of said time points in the presence of the candidate agent, and therefrom identifying an agent that alters mitochondrial function.

65. (Previously presented) The method of claim 64 wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the mitochondrion.

66. (Previously presented) The method of claim 64 wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium outside of the mitochondrion.

67. (Previously presented) The method of claim 64 wherein the step of contacting is repeated at least once.

68. (Previously presented) The method of claim 64 wherein the sample contains at least one compound that alters intracellular distribution of a calcium cation.

69. (Original) The method of claim 68 wherein the compound that alters intracellular calcium cation distribution is selected from the group consisting of thapsigargin and Ru360.

70. (Original) The method of claim 68 wherein the sample contains at least one compound that uncouples oxidative phosphorylation from ATP production.

71. (Previously presented) The method of claim 64 wherein the source of calcium cations is exogenous to the cell.

72. (Previously presented) The method of claim 64 wherein the sample contains at least one compound that uncouples oxidative phosphorylation from ATP production.

73. (Previously presented) The method of claim 64 wherein the cell comprises at least one polypeptide that is a Bcl-2 family member.

74. (Previously presented) The method of claim 64 wherein the cell expresses a gene encoding a polypeptide that regulates cytosolic calcium.

75. (Original) The method of claim 74 wherein the gene encodes a mitochondrial calcium uniporter.

76. (Original) The method of claim 74 wherein the gene is a transfected gene.

77. (Original) The method of claim 76 wherein the gene encodes a mitochondrial calcium uniporter.

78. (Previously presented) The method of claim 64 wherein the cell adheres to a solid substrate.

79. (Previously presented) The method of claim 64 wherein the cell is a non-adherent cell.

80. (Currently Amended) A method of identifying an agent that alters mitochondrial function, comprising:

(a) contacting, in each of a plurality of reaction vessels in a high throughput screening array,

(i) a biological sample comprising one or more isolated mitochondria and a calcium indicator molecule in a medium, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the biological sample;

(b) detecting in each reaction vessel the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity, wherein a decrease over time in the signal that is proportional to the level of calcium in the cytosol indicates mitochondrial calcium uniporter activity, and (ii) mitochondrial uncoupler or respiratory inhibitor activity, wherein an increase

over time in the signal that is proportional to the level of calcium in the cytosol without repeating step (a) indicates mitochondrial uncoupler or respiratory activity; and

(c) comparing the signal generated by the calcium indicator molecule at one or more of said time points in the absence of a candidate agent, to the signal generated by the calcium indicator molecule at one or more of said time points in the presence of the candidate agent, and therefrom identifying an agent that alters mitochondrial function.

81. (Currently Amended) A method of identifying an agent that alters mitochondrial function, comprising:

(a) contacting

(i) a biological sample comprising one or more isolated mitochondria and a calcium indicator molecule in a medium, under conditions that permit maintenance of mitochondrial membrane potential, with

(ii) a source of calcium cations,

wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the biological sample;

(b) detecting the signal generated by the calcium indicator molecule at a plurality of time points, under conditions that permit identification of (i) mitochondrial calcium uniporter activity, wherein a decrease over time in the signal that is proportional to the level of calcium in the cytosol indicates mitochondrial calcium uniporter activity, and (ii) mitochondrial uncoupler or respiratory inhibitor activity, wherein an increase over time in the signal that is proportional to the level of calcium in the cytosol without repeating step (a) indicates mitochondrial uncoupler or respiratory activity; and

(c) comparing the signal generated by the calcium indicator molecule at one or more of said time points in the absence of a candidate agent, to the signal generated by the calcium indicator molecule at one or more of said time points in the presence of the candidate agent, and therefrom identifying an agent that alters mitochondrial function.

82. (Original) The method of either claim 80 or claim 81 wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium in the mitochondrion.

83. (Original) The method of either claim 80 or claim 81 wherein the calcium indicator molecule is capable of generating a detectable signal that is proportional to the level of calcium outside of the mitochondrion.

84. (Original) The method of either claim 80 or claim 81 wherein the step of contacting is repeated at least once.

85. (Original) The method of either claim 80 or claim 81 wherein the sample contains at least one compound that alters distribution of a calcium cation in the sample.

86. (Original) The method of claim 85 wherein the compound that alters calcium cation distribution is selected from the group consisting of thapsigargin and Ru360.

87. (Original) The method of claim 85 wherein the sample contains at least one compound that uncouples oxidative phosphorylation from ATP production.

88. (Original) The method of either claim 80 or claim 81 wherein the isolated mitochondria are derived from a cell that comprises at least one polypeptide that is a Bcl-2 family member.

89. (Original) The method of either claim 80 or claim 81 wherein the isolated mitochondria are derived from a cell that expresses a gene encoding a polypeptide that regulates cytosolic calcium.

90. (Original) The method of claim 89 wherein the gene encodes a mitochondrial calcium uniporter.

91. (Original) The method of claim 89 wherein the gene is a transfected gene.

92. (Original) The method of claim 91 wherein the gene encodes a mitochondrial calcium uniporter.

93. (Previously presented) The method of any one of claims 1, 21, 64, 80 or 81 wherein subsequent to the step of contacting the biological sample with the source of calcium cations and prior to the step of comparing signals, the biological sample is contacted (i) with at least one compound that uncouples oxidative phosphorylation from ATP production, and (ii) with at least one agent that alters mitochondrial function.

94. (Original) The method of claim 93 wherein the agent that alters mitochondrial function is cyclosporin A.

95. (Original) The method of claim 93 wherein the agent that alters mitochondrial function is selected from the group consisting of cyclosporin A, rotenone, oligomycin, succinate and Bcl-2.

96. (Original) The method of claim 93 wherein the compound that uncouples oxidative phosphorylation from ATP production is selected from the group consisting of FCCP and CCCP.